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Notes on the Veliger Larvae and Settlement in the Anaspidean *Akera bullata*

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Abstract. *Akera bullata* is the only member of the Anaspidea to produce lecithotrophic larvae that settle in response to a variety of different substrata.

INTRODUCTION

In recent years, a great deal of research has been conducted on members of the Anaspidea, primarily investigating settlement and metamorphosis of the "sea hares," the Aplysiidae (Switzer-Dunlap & Hadfield, 1977; Strenth & Blankenship, 1978; Switzer-Dunlap, 1978; Otsuka et al., 1981; Switzer-Dunlap & Hadfield, 1981; Paige, 1986; Pawlik, 1989; Plaut et al., 1995). *Akera bullata* (Müller, 1776), however, has been largely overlooked. Phylogenetic studies have often included *A. bullata* to investigate the evolution of the Anaspidea and Opisthobranchia (Medina & Walsh, 2000; Klussmann-Kolb, 2003; Vonnemann et al., 2005; Klussmann-Kolb et al., 2008); despite this, little is know about its ecology. Populations of *A. bullata* have been found throughout the British Isles, and further afield from the Baltic and North Sea, the Atlantic Ocean, and the Mediterranean Sea: France, Italy, Spain, and Greece (Thompson, 1976; Thompson & Seaward, 1989).

Members of the Anaspidea typically produce planktotrophic veligers that spend approximately 1 mo feeding and developing before metamorphosis (Kriegstein et al., 1974; Switzer-Dunlap & Hadfield, 1977; Strenth & Blankenship, 1978; Switzer-Dunlap, 1978; Otsuka et al., 1981; Paige, 1986, 1988; Pawlik, 1989; Plaut et al., 1995). Anspideans metamorphose in response to stimuli produced by several different species of red, green, brown, and blue-green algae; often, the species inducing metamorphosis is the preferred food of the adult (Kriegstein et al., 1974; Switzer-Dunlap & Hadfield, 1977; Strenth & Blankenship, 1978; Switzer-Dunlap, 1978; Otsuka et al., 1981; Paige, 1986, 1988; Pawlik, 1989; Plaut et al., 1995). *Phyllaplysia taylori* is the exception: this species is unusual in that it undergoes direct development; metamorphosis occurs within the egg capsules, thus juveniles emerge from the egg mass (Bridges, 1975). Metamorphosis is similar for all species studied (Kriegstein et al., 1974; Switzer-Dunlap & Hadfield, 1977; Switzer-Dunlap, 1978; Paige, 1988). Once committed to metamorphosis, the veligers stop crawling and retract partially or completely into their shell. They remain in this state for most of metamorphosis, which can last between 2 and 4 days (Switzer-Dunlap, 1978). The velar cilia are shed, and the velum is reabsorbed. The oral tentacles (if present) start to develop at the site of the velum. Crawling is often resumed 1–2 days after the commencement of metamorphosis. The adult heart then develops, taking over in function from the larval heart (Switzer-Dunlap & Hadfield, 1977). During metamorphosis, feeding ceases despite the ability of the juveniles to rotate the radula and buccal mass (Kriegstein et al., 1974). Approximately 3 days after metamorphosis, the juveniles are able to bite and swallow (Kriegstein et al., 1974; Switzer-Dunlap & Hadfield, 1977; Switzer-Dunlap, 1978). This process is quicker for *B. leachii plei*, in which feeding was observed 1 day after metamorphosis (Paige, 1988). See Switzer-Dunlap & Hadfield (1977) and Switzer-Dunlap (1978) for further details regarding settlement cues and the morphological changes that occur during metamorphosis.

During June 2004, spawn from *A. bullata* was gathered from Langton Hive Point (UK grid reference SY606814), the Fleet, United Kingdom. The Fleet is a shallow, saline tidal lagoon covering an area of approximately 480 ha. It spans 12.5 km and is boarded by Chesil Beach northwest of Portland Bill, Dorset. Langton Hive Point is situated 8 km from the mouth of the lagoon and is predominantly brackish. Each spawn mass was placed into a sterile 100-mL glass beaker containing 50 mL of 0.45-μm-filtered sea water. In accordance with Switzer-Dunlap & Hadfield (1981), penicillin G and streptomycin sulfate were added to each beaker after every water change to create a final concentration of 60 μg mL^{-1} and 50 μg mL^{-1}, respectively. A 0.41-μm nylon mesh was floated on the meniscus to prevent the larvae rafting. Parafilm was used to cover the beakers to prevent evaporation. The

Table 1

Combinations of substratum, phytoplankton, or both used to investigate veliger settlement.

Substratum/ phytoplankton provided	No. replicates	Metamorphic success (%)*	Minimum larval period	Maximum larval period (days)
Chondrus crispus (C)	2	25 and 45	48 hr	25
Ulva lactuca (U)	2	60 and 50	5 days	32
Nemalion helminthoides	2	65 and 40	72 hr	25
Rhinomonas (R)	2	90 and 35	10 days	38
Tetraselmis (T)	2	10 and 35	15 days	31
Isochrysis (I)	2	95 and 75	48 hr	43
Chaetoceros (Ch)	2	25 and 45	25 days	43
Biofilm (B)	2	56 and 90	24 hr	17
Control	2	35 and 25	20 days	32
R + C	1	100	24 hr	16
R + U	1	56	72 hr	19
R + B	1	73	48 hr	16
T + C	1	43	5 days	13
T + U	1	70	5 days	19
T + B	1	37	24 hr	17
I + C	1	63	48 hr	19
I + U	1	57	24 hr	20
I + B	1	70	72 hr	21
Ch + C	1	57	24 hr	17
Ch + U	1	80	24 hr	24
Ch + B	1	33	5 days	10

* The two numbers given for metamorphic success are from replicates A and B, respectively.

water was changed on alternate days. The beakers were kept in a constant temperature room (18–20°C), with a light/dark photoperiod of 12:12 hr. Once hatched, 30 healthy veligers were removed using a Pasteur pipette and transferred to sterile 60-mL plastic containers. Each vessel contained a different substratum to investigate settlement (Table 1). The juveniles were not reared to sexual maturity.

RESULTS AND DISCUSSION

Akera bullata veligers successfully hatched from spawn gathered from the Fleet. The embryonic period could not be accurately determined in this study, although Thorson (1946, cited by Thompson, 1976) stated it to be 30 days at 15°C or 20 days at 20°C. The uncleaved eggs measured 154.4 ± 4.0 µm (mean ± SD) in diameter, and there was only one egg per capsule. This corresponds with Thompson (1976) and Thompson & Seaward (1989) who both reported diameters between 156 and 170 µm. On hatching, *A. bullata* had a shell length of 255.1 ± 13.1 µm (mean ± SD), and they possessed eyes, a large yellow yolk store within the left digestive diverticulum (termed the liver by Thorson [1946]), a stomach, a hind gut terminating at the anus, a larval kidney, a metapodium, larval retractor muscles, velar lobes with cilia, statocysts, and an operculum. Other larval structures were difficult to identify due to the density of the yolk (Figure 1A, B). Based on the evidence presented here, we conclude that *A. bullata* exhibits lecithotrophic development (type 2 of Thompson, 1967) and possesses a shell-type 2 of Thompson (1961). This is in agreement with Thorson (1946) but is a contradiction to the record of *A. bullata* possessing a shell-type 1 (Thompson, 1976).

Despite being lecithotrophic, the veligers are fully competent plankton feeders (facultative planktotrophs). When provided with *Rhinomonas*, the veligers' stomachs became pigmented, and the newly settled juveniles were able to produce defensive purple ink when disturbed (indicating the assimilation of phycoerythrin as a result of *Rhinomonas* digestion). However, feeding on phytoplankton is not necessary for metamorphosis, as has been recorded for other species of opisthobranchs but not for any species of the Anaspidea.

In agreement with the findings of Thorson (1946), shell growth did not occur during the planktonic stage. Metamorphic competence was attained once the propodium had inflated, in some veligers this occurred within 24 hr of hatching. Settlement and consequently metamorphosis occurred within 43 days after hatching, depending on the substratum, phytoplankton provided, or both (Table 1). Metamorphosis followed a pattern similar to that of other anaspideans. Most individuals had made the transition from swimming veliger to crawling juvenile between 6 and 12 hr after initial settlement. Metamorphosis in *A. bullata* was never a stationary event, and crawling was frequently observed.

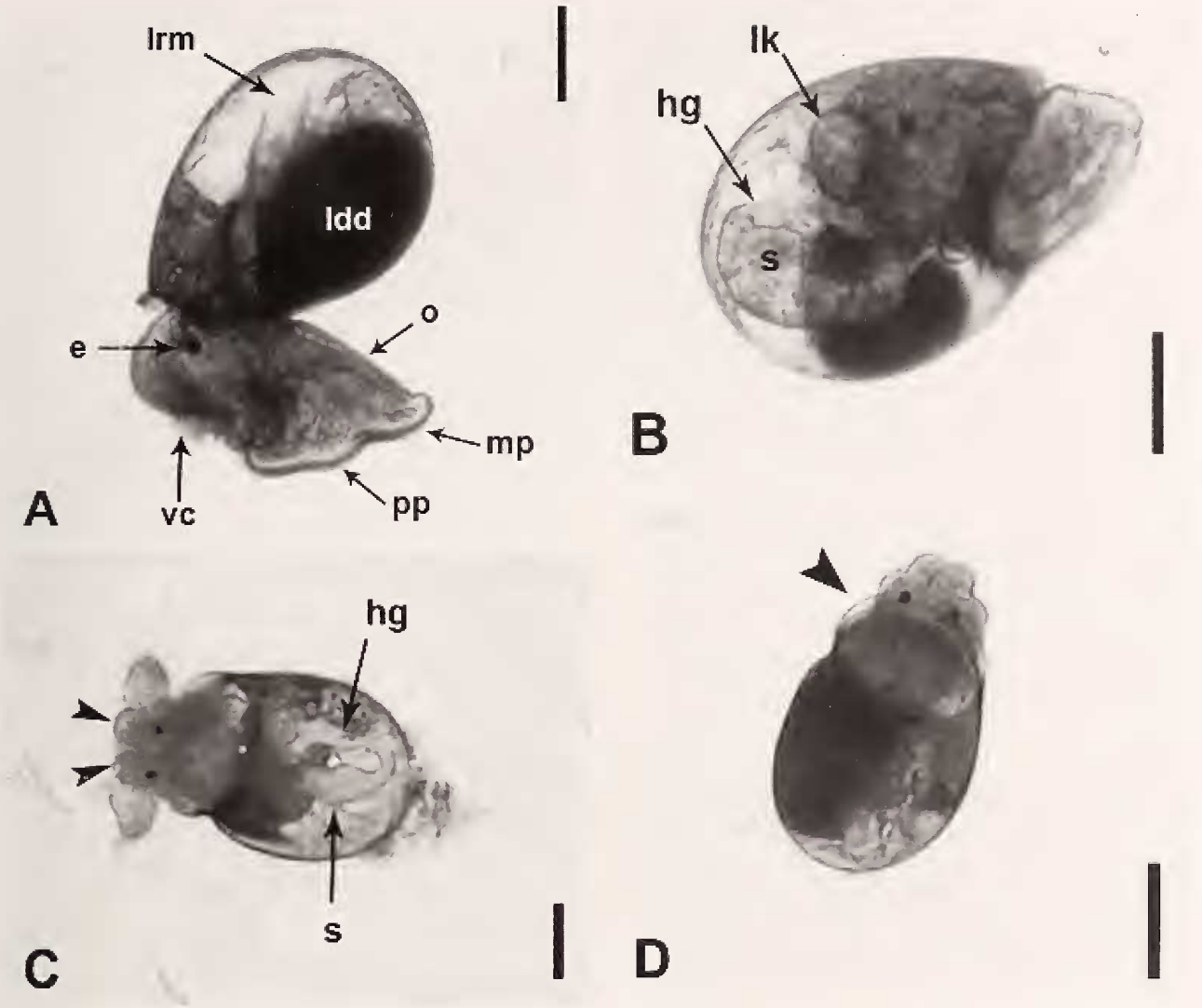

Figure 1. Newly hatched *Akera bullata* veligers (**A**, **B**) showing the type 2 shell; **C**, veliger undergoing metamorphosis; arrows point to the formation of the bilobed anterior region of the cephalic shield; **D**, metamorphosis is complete, although the operculum is still attached (arrow). Abbreviations: e, eye; hg, hind gut; lk, larval kidney; lrm, larval retractor muscle; mp, metapodium; o, operculum; pp, propodium; s, stomach; vc, velar cilia. Scale bars: A–D = 100 μm.

Initially, the velar cilia were absorbed, followed by the absorption of the velar lobes (Figure 1C). During the absorption of the velar lobes, the bilobed anterior end to the cephalic shield was formed (Figure 1D). This completed the veliger's transition into the benthic phase, after which it could no longer swim (until the parapodial lobes developed). The stage at which the operculum was lost (during metamorphosis) varied; after the loss of the operculum, the juvenile assumed the adult form. A larval heart was not visible at any time during the veliger stage. Statocysts were present but were difficult to distinguish due to the density of the tissues around the shell aperture. Movement of the buccal mass was observed within 48 hr of metamorphosis, although it is unknown whether food was swallowed during this time. After metamorphosis, the propodium formed a temporary pedal sole that then developed into the parapodial lobes that fold around the cephalic shield. Adult pigmentation appeared 7–18 days after metamorphosis. New shell growth initially formed as a collar extending from the original veliger shell. This progressively developed into whorls (Figure 1E). In some juveniles, a small finger-like projection was visible from the posterior end (Figure 1F). Although never reared

through to sexual maturity, the juvenile *A. bullata* were miniature replicas of the adults. The white pallial patterns of the juveniles as viewed through the shell were almost identical to those described by Thompson & Seaward (1989).

Members of the Anaspidea are generalist herbivores; however, in every species studied, successful postmetamorphic growth was restricted to only a few prey species. Switzer-Dunlap & Hadfield (1977) investigated the settlement preferences of several different species of aplysiids. The veligers of *Aplysia juliana* settled in response to the green algae *Ulva fasciata* and *Ulva reticulata*. *Aplysia dactylomela* veligers were not as specific and settled in response to *Laurencia*, *Chondrococcus*, *Gelidium*, *Martensia*, *Polysiphonia*, and *Spyridia* spp. However, *Laurencia* induced the greatest numbers of veligers to metamorphose. The larvae of *Dolabella auricularia* also metamorphosed in response to a variety of different algae: *Laurencia*, *Amansia*, *Spyridia*, *Sargassum*, and an unidentified mat-forming blue-green alga. Despite the range of metamorphic inducers, the juveniles of this species initially only consumed diatoms and blue-green algae. As they aged, their preferred diet changed to a mixture of *Spyridia*, *Acanthophora*, and *Laurencia*. *Stylocheilus longicauda* metamorphosed in response to *Lyngbya majuscula*, *Acanthophora*, and *Laurencia*, although only *L. majuscula* induced postlarval growth. The species that resulted in the greatest postmetamorphic survivorship in the study by Switzer-Dunlap & Hadfield (1977) were the species on which the adults were typically found. They did note however that for all species without a substrate no metamorphosis occurred. The veligers of *A. brasiliana* were induced to metamorphose by contact with *Callithamnion* and *Polysiphonia*; however, the greatest metamorphic success occurred on the latter species (Strenth & Blankenship, 1978). Pawlik (1989) discovered that *Aplysia californica* metamorphosed when exposed to any one of 18 different species of algae and in control dishes with no stimulus. Despite their indiscriminate settlement, they required a diet of either *Laurencia pacifica* or *Plocamium cartilagineum* for postlarval development (Pawlik, 1989). *Aplysia oculifera* metamorphosed in response to six of 12 macroalgal species tested by Plaut et al. (1995). None of the veligers metamorphosed under control conditions.

Akera bullata is known to be herbivorous. Thompson & Seaward (1989) documented *Enteromorpha* and possibly *Zostera* roots as prey. Morton & Holme (1955) found *A. bullata* grazing *Ulva* in Plymouth and also recorded it as a deposit feeder; in this study, adults also were found to graze a variety of different red and green algae and on the leaves of *Zostera* spp. It is not surprising therefore that *A. bullata* will settle on a variety of different substrata, including a bacterial biofilm, several species of phytoplankton, and algae. Similarly to the newly settled juveniles of *D. auricularia* (Switzer-Dunlap & Hadfield, 1977), all *A. bullata* juveniles were observed consuming biofilms. As they grew larger, the juveniles provided with *Ulva lactuca* were seen to consume the thin fronds, but never during this study were juveniles observed feeding upon *Choudrus crispus* or *Nemalion helminthoides*. It is likely that they had not reached a size that would have enabled them to consume the thick fronds. For the latter two conditions, the juveniles were only observed grazing on biofilms formed on the surface of the container and fronds.

The adoption of lecithotrophy may have profound implications on the ecology of *A. bullata*, particularly within an enclosed habitat such as the Fleet. Previous studies investigating British populations of the lecithotrophic nudibranch *Adalaria proxima*, which is able to undergo metamorphosis within 1–2 days after hatching (Thompson, 1958; Kempf & Todd, 1989; Lambert & Todd, 1994), were found to have significant differentiation over 100 km (Todd et al., 1998; Lambert et al., 2003). The short larval duration combined with a behavioral constraint were both implemented in its reduced dispersal (Todd et al., 1998; Lambert et al., 2003). A similar situation may be occurring between populations of *A. bullata* within the Fleet and elsewhere; however, further investigation is required to substantiate this claim.

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